Biochemical Diagnosis of Chromaffin Cell Tumors in Patients at High and Low Risk of Disease: Plasma versus Urinary Free or Deconjugated -Methylated Catecholamine Metabolites

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Abstract: BACKGROUND Measurements of plasma or urinary metanephrines are recommended for diagnosis of pheochromocytoma and paraganglioma (PPGL). What test offers optimal diagnostic accuracy for patients at high and low risk of disease, whether urinary free metanephrines offer advantages over deconjugated metanephrines, and what advantages are offered by including methoxytyramine in panels all remain unclear. METHODS A population of 2056 patients with suspected PPGLs underwent prospective screening for disease using mass spectrometric-based measurements of plasma free, urinary deconjugated, and urinary free metanephrines and methoxytyramine. PPGLs were confirmed in 236 patients and were excluded in others on follow-up evaluation. RESULTS Measurements of plasma free metabolites offered higher (< 0.01) diagnostic sensitivity (97.9%) than urinary free (93.4%) and deconjugated (92.9%) metabolites at identical specificities for plasma and urinary free metabolites (94.2%) but at a lower ($<$ 0.005) specificity for deconjugated metabolites (92.1%). The addition of methoxytyramine offered little value for urinary panels but provided higher (< 0.005) diagnostic performance for plasma measurements than either urinary panel according to areas under ROC curves (0.991 vs 0.972 and 0.964). Diagnostic performance of urinary and plasma tests was similar for patients at low risk of disease, whereas plasma measurements were superior to both urinary panels for high-risk patients. CONCLUSIONS Diagnosis of PPGLs using plasma or urinary free metabolites provides advantages of fewer false-positive results compared with commonly measured deconjugated metabolites. The plasma panel offers better diagnostic performance than either urinary panel for patients at high risk of disease and, with appropriate preanalytics, provides the test of choice. Measurements of methoxytyramine in urine show limited diagnostic utility compared with plasma.

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Biochemical Diagnosis of Chromaffin Cell Tumors in Patients at High and Low Risk of Disease: Plasma versus Urinary Free or Deconjugated *O*-Methylated Catecholamine Metabolites

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BACKGROUND: Measurements of plasma or urinary metanephrines are recommended for diagnosis of pheochromocytoma and paraganglioma (PPGL). What test offers optimal diagnostic accuracy for patients at high and low risk of disease, whether urinary free metanephrines offer advantages over deconjugated metanephrines, and what advantages are offered by including methoxytyramine in panels all remain unclear.

METHODS: A population of 2056 patients with suspected PPGLs underwent prospective screening for disease using mass spectrometric-based measurements of plasma free, urinary deconjugated, and urinary free metanephrines and methoxytyramine. PPGLs were confirmed in 236 patients and were excluded in others on follow-up evaluation.

RESULTS: Measurements of plasma free metabolites offered higher ($P < 0.01$) diagnostic sensitivity (97.9%) than urinary free (93.4%) and deconjugated (92.9%) metabolites at identical specificities for plasma and urinary free metabolites (94.2%) but at a lower ($P < 0.005$) specificity for deconjugated metabolites (92.1%). The addition of methoxytyramine offered little value for urinary panels but provided higher ($P < 0.005$) diagnostic performance for plasma measurements than either urinary panel according to areas under ROC curves (0.991 vs 0.972 and 0.964). Diagnostic performance of urinary and plasma tests was similar for patients at low risk of disease, whereas plasma measurements were superior to both urinary panels for high-risk patients.

CONCLUSIONS: Diagnosis of PPGLs using plasma or urinary free metabolites provides advantages of fewer false-positive results compared with commonly measured deconjugated metabolites. The plasma panel offers better diagnostic performance than either urinary panel for patients at high risk of disease and, with appropriate preanalytics, provides the test of choice. Measurements of methoxytyramine in urine show limited diagnostic utility compared with plasma.

Pheochromocytomas and paragangliomas (PPGLs)[11] include adrenal and extraadrenal chromaffin cell tumors as well as head and neck paragangliomas. The former catecholamine-producing chromaffin cell tumors must be considered among large numbers of patients with hypertension and symptoms of catecholamine excess, as well as for patients at higher risk of disease because of a hereditary predisposition, the finding of an incidentaloma, or a history of PPGLs *(1–3)*. Biochemical testing is crucial for diagnosis of the tumors. To this end, current clinical practice guidelines stipulate with a high level of evidence that biochemical screening for PPGLs should include measurements of plasma free or urinary fraction-

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[11] Nonstandard abbreviations: PPGL, pheochromocytoma and paraganglioma; UC, upper cutoff; AUC, area under the ROC curve.

ated metanephrines (i.e., normetanephrine and metanephrine), with no recommendation concerning preference of either test *(4)*.

Although some studies have indicated higher accuracy of plasma free than urinary fractionated metanephrines for diagnosis of PPGLs *(5, 6)*, others have not *(7, 8)*, and all had limitations. None involved head-to-head comparisons by LC-MS/MS. After introduction by Lagerstedt et al. in 2002 *(9)*, LC-MS/MS now provides the method of choice for measurements of plasma free metanephrines as reflected by an international quality assurance program *(10)*, in which 92% of all participants now use the technology. Among all participating laboratories, two-thirds also measure methoxytyramine, the metabolite of dopamine. Although measurements of plasma methoxytyramine appear useful *(11, 12)*, the comparative utility of urinary measurements of methoxytyramine remains unclear. In this report, the term "metanephrines" is restricted to covering normetanephrine and metanephrine.

It has been suggested that plasma measurements of metanephrines suffer from an overabundance of false-positive results, restricting suitability of the test to patients at high risk for PPGLs *(13)*. That assertion has since been clarified by findings that sampling blood in the seated position rather than the recommended supine position results in a 6-fold increase in false-positive results *(14)*. It remains unclear whether diagnostic accuracy of plasma and urinary tests differs among populations according to differing pretest prevalences and, thus, risk of PPGLs.

Another consideration in use of tests concerns whether measurements of metanephrines in urine should continue to use an acid-hydrolysis deconjugation step or whether measurements of free metanephrines, without that step, might offer advantages *(15–17)*. At least for plasma measurements, the free metanephrines provide superior diagnostic performance compared with the deconjugated metanephrines *(18)*. This is because the deconjugated metanephrines mainly reflect sulfate conjugates produced by a specific sulfotransferase enzyme localized to gastrointestinal tissues where nearly half of all norepinephrine is produced and metabolized within the body *(19)*. It remains unclear whether these differences also offer advantages to urinary free compared with deconjugated metanephrines. Substantial production of dopamine in gastrointestinal tissues *(20)* may similarly provide advantages to use of free methoxytyramine over the deconjugated metabolite, but this is also unclear.

Based on the above considerations, the present study addressed the hypothesis that measurements in plasma and urine of the free forms of catecholamine *O*-methylated metabolites should offer superior performance for diagnosis of PPGLs than routinely used urinary deconjugated metabolites. We also examined differences in diagnostic performance of the 3 test panels with and without methoxytyramine and whether test performance might differ according to the basis of testing and relative risk of disease. As initiated in 2011 under a multicenter prospective protocol, the study aimed to accrue at least 2000 patients, including 200 with PPGLs. The study involved follow-up to establish a final diagnosis; all measurements were by LC-MS/MS; and for the plasma test, all patients were sampled in the supine position according to clinical practice guidelines *(4)*.

Study Design and Methods

STUDY POPULATION

The study population included 2056 patients (1011 male) with a median age of 53 years (range, 10–93 years) screened for PPGLs under a multicenter prospective protocol as detailed in the Methods section of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol64/issue11. In brief, enrollment of patients at 6 European tertiary medical centers followed 4 criteria establishing suspicion or risk for PPGLs: (*a*) signs and/or symptoms of catecholamine excess; (*b*) hereditary risk of PPGL; (*c*) findings of an incidentaloma; or (*d*) history of PPGL. The study included multiple phases, from screening to follow-up, and was conducted according to ethics committee-approved clinical protocols and standard operating procedures available online (https://pmt-study.pressor.org). A reference population of 351 normotensive (132 male) and 239 hypertensive (129 male) volunteers with a median age of 42 years (range, 18–82 years) was also included for establishing reference intervals as covered in the Methods section of the online Data Supplement. All participants provided written informed consent.

TUMOR DIAGNOSIS AND FOLLOW-UP

Initial detection of PPGLs was based in most cases on positive biochemical test results, but follow-up was required to further confirm or exclude disease in other cases (see the Methods section of the online Data Supplement). PPGLs in 5 patients were diagnosed at follow-up >1 year after initial biochemical testing. Patients presenting solely with nonchromaffin cell head and neck paragangliomas were excluded from the analysis. In this way, 236 patients were finally diagnosed with chromaffin cell tumors with confirmation achieved by pathological examination of surgically resected tumors (n = 207) or by functional imaging evidence of disease in cases that were not surgically resected because of metastatic involvement or refusal of surgery (n = 29). Follow-up information to exclude PPGLs was available in 97% (1768 of 1820) of patients, of which 56% of cases required an interval of >2 years from study entry to exclude disease. As detailed in the Methods section of the online Data

Supplement, shorter durations could be accepted in other patients, particularly those who underwent adrenalectomies, imaging studies, or endocrine testing to establish an alternative diagnosis.

BLOOD AND URINE COLLECTIONS

For blood sampling, patients were required to fast overnight and to maintain a fully supine position for 30 min up until the time of the blood draw (10 mL). Blood samples collected into heparinized tubes were placed on ice or cool pads at 4 °C before centrifugation to separate plasma. On the final day of collection, 24-hour urine specimens were returned to study centers. Urine volumes were then determined and samples aliquoted. All specimens collected at different study centers were transported on dry ice to the central analytical laboratory where samples were assayed.

BIOCHEMICAL ANALYSES

Measurements of plasma and urinary metanephrines (normetanephrine, metanephrine) and methoxytyramine were performed at a single laboratory using LC-MS/MS *(21, 22)*. For urine specimens, measurements were performed with and without an acid hydrolysis step, the latter for measurements of the free metabolites and the former to convert sulfate-conjugated metabolites into the free form for measurements of combined free plus sulfate conjugated (deconjugated) metabolites. As outlined in the Methods section of the online Data Supplement, data from the reference population were used to establish upper cutoffs (UCs) of reference intervals for urinary metabolites and to validate those previously established for plasma metabolites, including age-specific UCs for normetanephrine. All UCs are specified in the Methods section of the online Data Supplement.

DATA ANALYSES

Diagnostic sensitivity was estimated from the percentage of true-positive results among both true-positive and false-negative results for patients with PPGLs. Diagnostic specificity was estimated from the percentage of true-negative results among both true-negative and false-positive results. ROC curves were constructed using logistic regression with comparisons of areas under the ROC curves (AUCs) to assess differences in diagnostic test performance. Positive predictive values (posttest probability of a positive result) were calculated across prevalence rates (pretest probability) using positive likelihood ratios. Curves relating the prevalence rates and positive predictive values were constructed according to relative increases above cutoffs. Statistical analyses were done using the JMP statistics software package (SAS Institute).

Results

PLASMA CONCENTRATIONS AND URINARY OUTPUTS OF *O*-METHYLATED METABOLITES

Plasma concentrations and urinary outputs of catecholamine *O*-methylated metabolites were similar in the reference population and patients screened for PPGLs in whom disease was excluded (Table 1). Methoxytyramine for both groups showed completely different proportions relative to normetanephrine and metanephrine in plasma compared with urine. Plasma concentrations of free methoxytyramine were much lower ($P < 0.0001$), <20% those of normetanephrine and metanephrine. In contrast, urinary outputs of free methoxytyramine were 32% to 53% higher ($P < 0.0001$) than outputs of free normetanephrine and metanephrine. For the deconjugated metabolites, urinary outputs of methoxytyramine were similar to normetanephrine but 72% to 81% higher ($P < 0.0001$) than metanephrine, again a divergent pattern compared with plasma.

Among patients with PPGLs, plasma and urinary free normetanephrine showed similar 9.6- to 10.4-fold increases above values in patients without tumors, compared with a smaller ($P < 0.0001$) 5.8-fold increase for urinary deconjugated normetanephrine (Table 1). Metanephrine showed similar 3.9- to 4.0-fold increases for all 3 tests, whereas methoxytyramine showed a larger ($P < 0.001$) 2.9-fold increase for the plasma test compared with 1.5- to 1.6-fold increases for urine tests.

As a group, patients screened because of an incidentaloma, hereditary risk, or previous disease history had a 3-fold higher ($P < 0001$) prevalence of PPGLs than patients tested because of signs and symptoms of presumed catecholamine excess (see Table 4 in the online Data Supplement). Plasma concentrations and urinary outputs of free and deconjugated normetanephrine and metanephrine were consistently higher ($P < 0.05$) among patients with tumors of the low than high PPGL prevalence group (see Table 5 in the online Data Supplement).

FALSE-NEGATIVE RESULTS

Among the 236 patients with confirmed PPGLs, there were 5 patients with false-negative results for plasma free metabolites, including 2 with negative results for both urinary panels and 2 others with negative results for urinary free metabolites (see Table 1 in the online Data Supplement). Sixteen other patients had false-negative results for urinary free or deconjugated metabolites but positive results for plasma free metabolites, including 11 with negative free metabolite results, 14 with negative results for deconjugated metabolites, and 9 with negative results for both urinary panels (see Table 2 in the online Data Supplement).

The 21 patients with false-negative results for plasma free, urinary free, or urinary deconjugated metab-

Table 1. Plasma concentrations (medians and ranges) and urinary outputs of catecholamine *O*-methylated metabolites in the reference population and patients with and without PPGLs.

Test panel	Reference population[a]	No PPGL	PPGL
Plasma free metabolites	n = 586[a]	n = 1820	n = 236
Normetanephrine, pg/mL	62 (18–201)	67 (11–365)	642 (45–25 444)
Metanephrine, pg/mL	29 (5–89)	30 (0.2–145)	120 (5–6889)
Methoxytyramine, pg/mL	4.9 (1.4–17.6)	4.8 (0.4–36.7)	14.1 (0.6–11 444)
Urine free metabolites	n = 580[a]	n = 1756[b]	n = 226[b]
Normetanephrine, μg/day	21 (4–100)	22 (1–170)	229 (9–3478)
Metanephrine, μg/day	18 (2–61)	16 (0.2–172)	64 (1–3547)
Methoxytyramine, μg/day	33 (4–136)	34 (2–212)	50 (8–3202)
Urine deconjugated metabolites	n = 581[a]	n = 1757[b]	n = 226[b]
Normetanephrine, μg/day	189 (41–803)	212 (26–2678)	1239 (172–21 850)
Metanephrine, μg/day	105 (17–446)	108 (1–991)	419 (9–14 946)
Methoxytyramine, μg/day	188 (52–2185)	197 (20–2990)	323 (58–13 031)

[a] Inclusion of the reference population in the table is to provide a comparison with patients without PPGLs. Specified ranges do not indicate the reference intervals that were used, which are supplied in the online Data Supplement. Among the 590 subjects of the reference population, measurements of plasma concentrations and urinary outputs of metabolites were not possible in up to 10 patients.

[b] Urinary measurements were not possible in up to 64 of the 1820 patients without PPGLs and 10 patients with PPGLs. To convert pg/mL to pmol/L and μg/day to nmol/day, divide values for normetanephrine, metanephrine, and methoxytyramine by 0.1832, 0.1972, and 0.1672, respectively.

olites showed numerous differences in how their disease presented compared with the other 215 patients with PPGLs. Only 1 of the 21 patients with false-negative results was tested because of signs and symptoms, a lower ($P = 0.0013$) proportion than the group with true-positive results (5% vs 40%). A larger ($P < 0.0001$) proportion of patients with false-negative than true-positive results was tested because of hereditary risk or a history of PPGLs (71% vs 23%). Patients with false-negative results also were characterized by a higher prevalence of metastatic disease (48% vs 13%; $P = 0.0005$), extraadrenal tumors (43 vs 16%; $P = 0.0053$), and tumors without production of epinephrine as characterized by no increase in plasma metanephrine (95% vs 42%; $P < 0.0001$).

As outlined in the Methods section of the online Data Supplement, all 5 patients in whom PPGLs were identified after follow-up of >1 year belonged to the group of 21 patients with false-negative results.

TRUE-POSITIVE VS FALSE-POSITIVE RESULTS

Using UCs optimized to maintain high diagnostic sensitivity with minimal loss of specificity (see Methods section in the online Data Supplement), proportions of true-positive results were 10% and 6% higher ($P < 0.005$) for plasma and urinary free normetanephrine, respectively, than for urinary deconjugated normetanephrine (Fig. 1, A, D, and G). Conversely, proportions of false-positive results were 28% and 23% lower ($P < 0.05$) for plasma and urinary free normetanephrine, respectively, than for urinary deconjugated normetanephrine. Proportions of true- and false-positive results for metanephrine showed only small differences among test panels (Fig. 1, B, E, and H).

Among all metabolites, methoxytyramine showed the largest differences in proportions of true-positive results for the 3 test panels (Fig. 1, C, F, and I). True-positive results for urinary deconjugated methoxytyramine were observed in 8% of patients with PPGLs compared with proportions nearly 2-fold higher ($P = 0.0003$) for urinary free methoxytyramine and 5.4-fold higher ($P < 0.0001$) for plasma free methoxytyramine. Proportions of true-positive results were also 2.8-fold higher ($P < 0.0001$) for plasma free than urinary free methoxytyramine (43.3% vs 15.5%).

DIAGNOSTIC SENSITIVITY AND SPECIFICITY

Diagnostic sensitivity of the plasma panel was higher than for panels of urinary free and deconjugated metabolites both with ($P < 0.01$) and without ($P < 0.05$) inclusion of methoxytyramine (Table 2). Inclusion of methoxytyramine had little effect on diagnostic sensitivities of urinary panels but returned positive results for plasma measurements in 3 patients with otherwise false-negative results. Diagnostic specificities, with and without inclusion of methoxytyramine, were higher ($P < 0.02$) for panels of plasma and urinary free metabolites than deconjugated metabolites.

Differences in diagnostic performance between plasma and urinary panels diverged after separation of

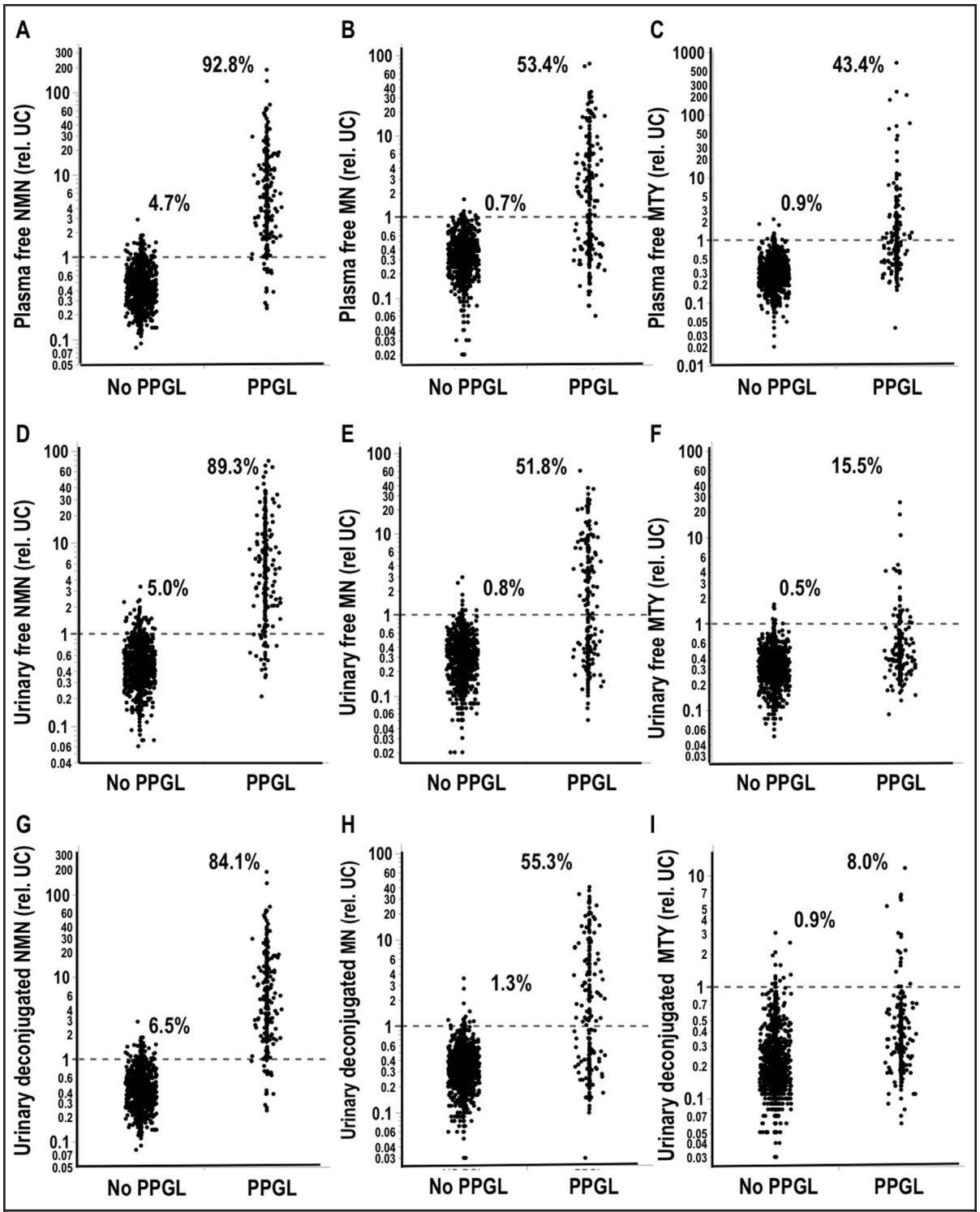

Fig. 1. Plasma concentrations (A–C) and 24-h urinary outputs of free (D–F) and deconjugated (G–I) normetanephrine (A, D, and G), metanephrine (B, E, and H), and methoxytyramine (G–I) in patients with and without PPGLs.

All data are shown relative to UCs of reference intervals as shown by dashed horizontal lines. Percentage values in each panel show proportions of positive results for each measurement.

Table 2. Diagnostic sensitivities and specificities of plasma and urinary panels of *O*-methylated metabolites.

Group[a]	Sensitivity, %	Specificity, %
All patients (NMN and MN)		
Plasma free	96.6 (228/236)[b]	94.9 (1727/1820)[c]
Urinary free	92.9 (210/226)	94.5 (1660/1756)[c]
Urinary deconjugated	92.9 (210/226)	92.8 (1630/1757)
All patients (NMN, MN, and MTY)		
Plasma free	97.9 (231/236)[b]	94.2 (1714/1820)[c]
Urinary free	93.4 (211/226)	94.2 (1655/1756)[c]
Urinary deconjugated	92.9 (210/226)	92.1 (1619/1757)
High pretest prevalence (NMN, MN, and MTY)		
Plasma free	96.7 (145/150)[b]	92.8 (569/613)
Urinary free	89.6 (129/144)	92.8 (542/583)
Urinary deconjugated	89.5 (128/143)	91.8 (536/584)
Low pretest prevalence (NMN, MN, and MTY)		
Plasma free	100 (86/86)	94.9 (1145/1207)[c]
Urinary free	100 (82/82)[d]	95.0 (1114/1173)[c]
Urinary deconjugated	98.8 (82/83)[d]	92.3 (1083/1173)

[a] Data are shown for all patients (All patients) and patients tested because of signs and symptoms (Low pretest prevalence) or all those tested because of hereditary risk, previous history of PPGL, or an incidentaloma (High pretest prevalence). Data for all patients includes results for plasma normetanephrine (NMN) and metanephrine (MN) considered together (NMN and MN) or with additional measurements of methoxytyramine (MTY) for all 3 metabolites (NMN, MN, and MTY). NMN, normetanephrine; MN, metanephrine; MTY, methoxytyramine.
[b] $P < 0.05$, higher sensitivity of plasma than both urinary panels.
[c] $P < 0.02$, higher specificity of panels for plasma and urinary free metabolites than urinary deconjugated metabolites.
[d] $P < 0.01$, higher sensitivity for low than high prevalence group.

patients into the 2 groups at high and low risk of PPGLs. Diagnostic sensitivities for both urinary panels, but not the plasma panel, were higher ($P < 0.01$) in patients at lower risk of disease tested because of signs and symptoms compared with those at higher risk with incidentalomas, a previous disease history, or an underlying mutation. Accordingly, only patients with a high pretest prevalence of PPGLs showed a higher ($P < 0.02$) diagnostic sensitivity of the plasma than both urinary panels. In contrast, only patients with a low pretest prevalence of PPGLs were those in whom diagnostic specificity was higher ($P < 0.01$) for panels of plasma and urinary free than deconjugated metabolites.

ROC CURVES

AUCs for individual metabolites showed the most substantial differences for methoxytyramine, which for the plasma panel showed higher ($P < 0.0001$) diagnostic performance than both urinary panels (Table 3). For normetanephrine, areas were higher for plasma ($P = 0.0014$) and urinary free ($P = 0.0162$) than deconjugated measurements, whereas areas were similar for metanephrine. With models using both normetanephrine and metanephrine, the plasma panel provided a higher ($P = 0.0021$) AUC than for measurements of urinary deconjugated but not urinary free metabolites (Fig. 2 and Table 3). With the addition of methoxytyramine, diagnostic performance of the plasma panel exceeded that for both panels of urinary free ($P = 0.0036$) and deconjugated ($P = 0.0005$) metabolites. With separate examination of patients with low and high pretest prevalence of PPGLs, differences in diagnostic performance between plasma and urinary tests disappeared for patients in the low pretest prevalence group but remained significant for the high prevalence group.

POSITIVE PREDICTIVE VALUES

After correction of posttest probabilities of PPGLs according to differences in test performance for patients at low and high risk of PPGLs (see Results section in the online Data Supplement), posttest probabilities of PPGLs not only varied according to pretest prevalences of disease but also according to the test panel and extent of increases of metabolites above UCs (Fig. 3). Thus, at high pretest prevalences of ≥5%, results for the plasma panel that were ≥2-fold above UCs indicated a 99% probability of disease in 80% of patients with PPGLs compared with 96% and 88% probabilities in 75.0% and 66.4% of patients for panels of urinary free and deconjugated metabolites, respectively. At lower pretest preva-

Table 3. AUCs (with 95% CI) for tests of plasma free vs urinary free and deconjugated metabolites according to individual metabolites and combinations of metabolites for all patients and patient groups with high and low pretest prevalences of PPGLs.

	Plasma free metabolites	Urinary free metabolites	Urinary deconjugated metabolites
All patients			
NMN[a]	0.971 (0.953–0.982)[b]	0.962 (0.941–0.976)[b]	0.946 (0.926–0.961)
MN[c]	0.788 (0.740–0.821)	0.773 (0.729–0.812)	0.788 (0.744–0.826)
MTY[d]	0.877 (0.849–0.901)[e,f]	0.713 (0.673–0.750)	0.722 (0.685–0.756)
NMN and MN	0.984 (0.971–0.992)[b]	0.973 (0.955–0.984)	0.964 (0.944–0.977)
NMN, MN, and MTY	0.991 (0.985–0.995)[e,f]	0.972 (0.954–0.983)	0.964 (0.944–0.978)
High pretest prevalence			
NMN and MN	0.971 (0.949–0.983)[b]	0.958 (0.930–0.975)	0.945 (0.913–0.966)
NMN, MN, and MTY	0.981 (0.969–0.989)[e,g]	0.956 (0.927–0.974)	0.944 (0.911–0.965)
Low pretest prevalence			
NMN and MN	0.999 (0.996–1.000)	0.999 (0.996–1.000)	0.997 (0.992–0.999)
NMN, MN, and MTY	1.000 (0.999–1.000)	0.999 (0.995–1.000)	0.998 (0.993–0.999)

[a] Normetanephrine.
[b] $P < 0.02$, higher than urinary deconjugated metabolites.
[c] Metanephrine.
[d] Methoxytyramine.
[e] $P < 0.001$, higher than urinary deconjugated metabolites.
[f] $P < 0.005$, higher than urinary free metabolites.
[g] $P < 0.02$, higher than urinary free metabolites.

lences of disease, posttest probabilities of PPGLs showed larger differences between test panels associated with smaller differences in proportions of patients with positive test results. Thus, at a 0.5% pretest prevalence of disease, results for the plasma panel that were ≥2-fold above UCs indicated a 76% probability of PPGLs in 98.8% of patients with tumors compared with 65% and 56% probabilities in 95.1% and 92.8% of patients for respective panels of urinary free and deconjugated metabolites.

Discussion

Using a fully prospective design with recruitment of >2000 patients, this study provides novel data and important updates about currently recommended screening tests for PPGLs. We show that measurements of plasma free metabolites provide a superior diagnostic test than routinely used urinary fractionated metabolites, both in terms of diagnostic sensitivity and specificity, as well as overall performance as manifest by AUCs. We also establish that measurements of the free metabolites in urine provide diagnostic advantages over the deconjugated metabolites, justifying phasing out the hydrolysis step in the latter test. Furthermore, although measurements of plasma free methoxytyramine can be useful for diagnosis of PPGLs, measurements in urine have negligible diagnostic value. Finally, the superiority of the plasma over urinary tests applies principally to patients tested because of high risk of PPGLs. For patients at lower risk, specifically those tested because of signs and symptoms of presumed catecholamine excess, urinary and plasma tests display similarly high performance. This implies that patients at high risk for PPGLs benefit the most from the plasma test. In contrast, plasma measurements provide minimal diagnostic advantages over urinary measurements for symptomatic patients at low risk for PPGLs.

Findings that the plasma free metabolites provide overall superior diagnostic performance compared with urinary deconjugated metabolites is not unexpected when considering the sources of catecholamines and the pathways of their metabolism *(19)*. Similarities and differences in diagnostic signal strengths of plasma free vs urinary free and deconjugated metabolites are thereby easily explained by differences in their relative tumoral and nontumoral sources (see Discussion section in the online Data Supplement). Irrespective of the underlying biology, the present findings clearly also establish for the first time that measurements of both urinary free and deconjugated methoxytyramine provide an inferior method to assess tumoral production of dopamine compared with plasma free methoxytyramine. This conclusion is consistent with other data indicating that urinary

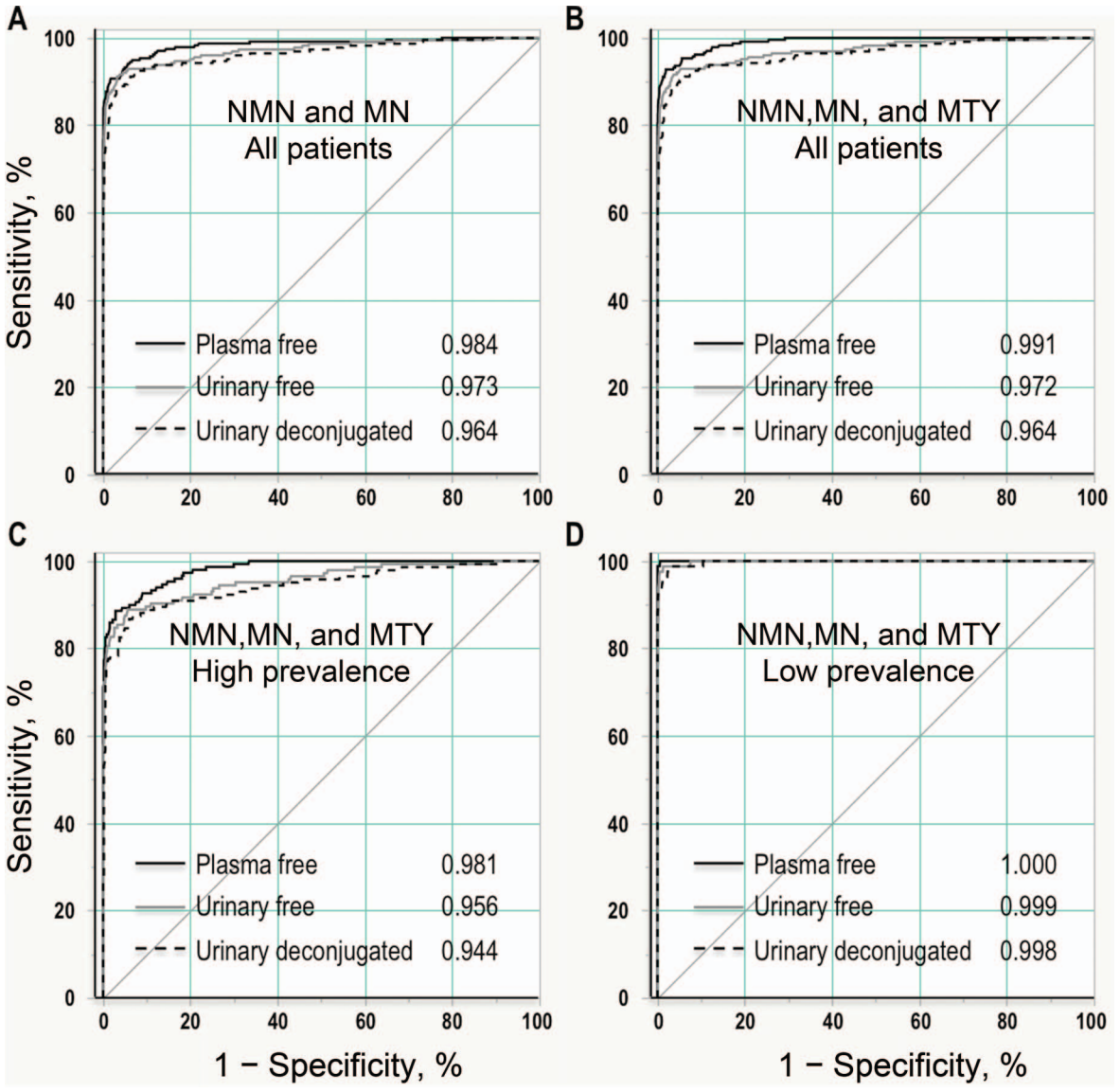

Fig. 2. ROC curves for plasma free, urinary free and urinary deconjugated *O*-methylated metabolites, constructed according to multivariable logistic regression model combinations of normetanephrine (NMN), metanephrine (MN), and methoxytyramine (MTY).

Models included all patients with and without PPGLs for NMN and MN in (A) and for NMN, MN, and MTY in (B). Models for all 3 metabolites but restricted to patients with a high pretest prevalence of PPGLs (i.e., tested because of genetic risk, previous history, or incidentaloma) are shown in (C), whereas those with a low pretest prevalence of PPGLs (i.e., tested because of signs and symptoms) are shown in (D). Areas under each of the 3 ROC curves are indicated in each panel.

methoxytyramine has limited utility for identifying dopamine-producing PPGLs *(23)*.

Although the present findings establish overall higher performance of plasma than urinary metabolites for diagnosis of PPGLs, this does not mean that urinary tests should be abandoned in favor of the plasma test. High diagnostic performance of the plasma test can only be achieved with an accurate method of measurement, appropriately established reference intervals, and strict adherence to preanalytical precautions of supine rest and, for plasma methoxytyramine, an overnight fast before blood sampling *(14, 24–26)*. Use of inaccurate immu-

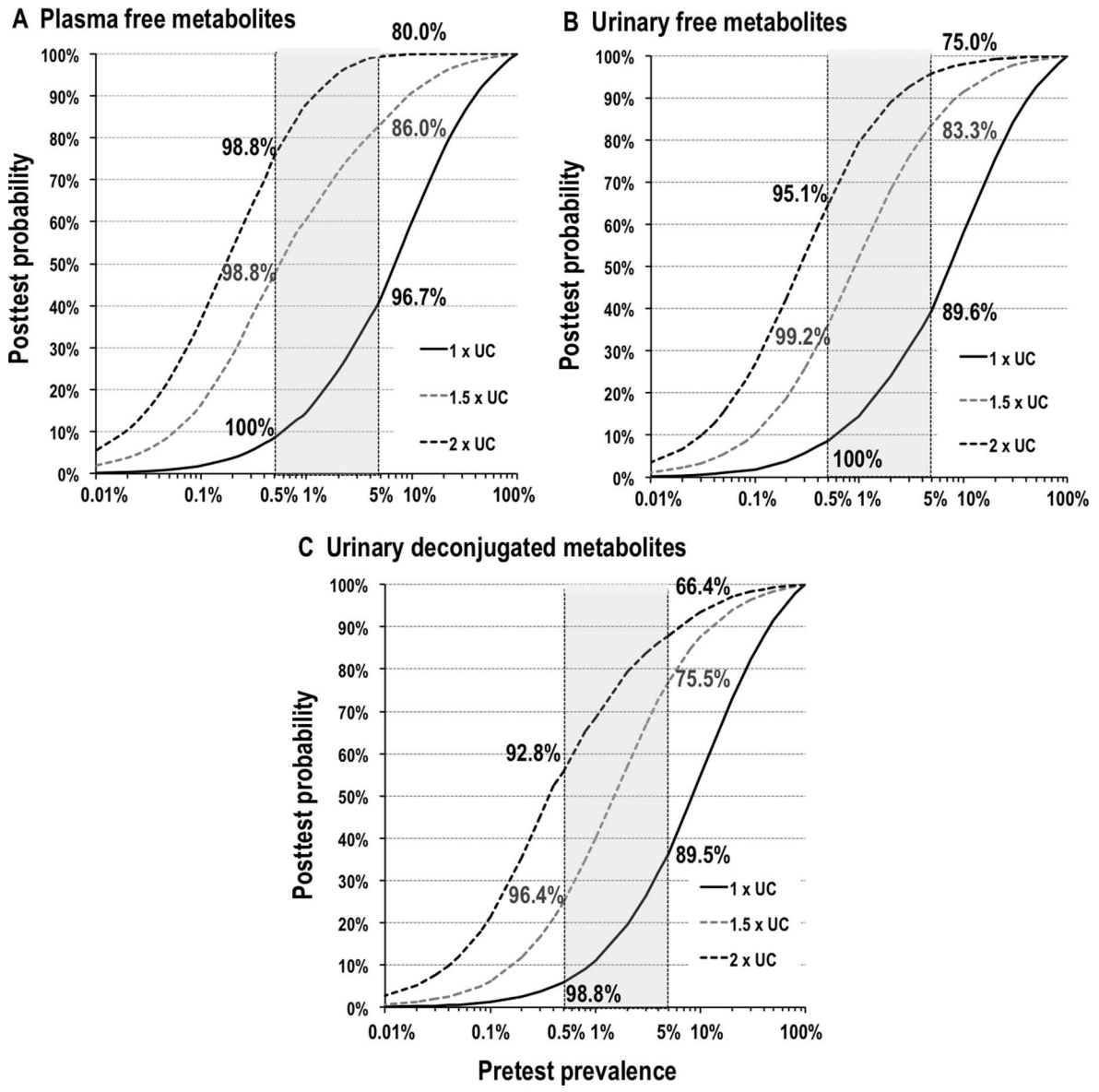

Fig. 3. Relationships of pretest prevalence of PPGLs vs posttest probability of the tumors according to tests of plasma free metabolites (A), urinary free metabolites (B), and urinary deconjugated metabolites (C).

Posttest probabilities are shown according to positive results above UCs (1 × UC), positive results with tests for any analyte ≥1.5-fold above UCs (1.5 × UC) and positive results within tests for any analyte ≥2-fold above UCs (2 × UC). Shaded areas serve to illustrate posttest probabilities at common pretest prevalences between 0.5% and 5%. Percent values adjacent to shaded areas of each panel represent proportions of positive results (i.e., diagnostic sensitivities) at the 3 cutoff levels (1, 1.5, and 2 × UC) at both high and low probabilities of PPGLs, which for values of 1 × UC are according to the data of Table 2.

noassays and inappropriately established reference intervals in one study resulted in >25% of patients with PPGLs returning negative results for the plasma test *(27)*. Even with accurate analytical methods, without supine rest and fasted sampling, diagnostic performance of the plasma test can be severely compromised to the same level or less than the urinary test *(8, 14)*. Diagnostic sensitivity can be particularly compromised with reference intervals established from seated rather than supine sampling *(25, 28)*. Thus, the urinary test may remain

preferable for centers that cannot achieve the correct combination of an accurate analytical method, appropriately established reference intervals, and adherence to recommended preanalytical precautions of blood sampling.

As further established, the urinary panels appear adequate for diagnosis of PPGLs among patients with signs and symptoms of presumed catecholamine excess who carry a relatively low pretest prevalence of disease. Because such patients are most likely to be first encountered at primary and secondary clinical care centers, use of urinary measurements may be most appropriate for such centers where adherence to the preanalytical precautions for blood sampling may be difficult.

For centers where urinary measurements remain preferable, the present study provides important new data to support phasing out measurements of deconjugated metanephrines in favor of free metanephrines. In particular, measurements of urinary free metanephrines provide improved diagnostic specificity and positive predictive value compared with deconjugated metanephrines. Lack of requirement for an acid hydrolysis step during sample preparation provides a further benefit. Because commercially available calibrators and quality control samples are provided with the metabolites almost completely present in the free form, another advantage of the free metabolites is that any variation in the efficiency of the acid hydrolysis step will not compromise the accuracy of results *(17, 24)*.

Although urinary tests appear sufficient for diagnosis of PPGLs among low-risk symptomatic patients, the plasma test is clearly preferable for patients at higher risk of disease, with inclusion of methoxytyramine being particularly important for patients with mutations of specific genes or for whom there is risk of recurrent or metastatic disease *(11, 29)*. Because such patients are those most usually screened at tertiary care centers, these are also the centers where the plasma panel is particularly important. With appropriate expert multidisciplinary teams, such centers provide the best location for personalized and correct implementation and interpretation of laboratory tests to appropriately guide patient treatment *(4, 30, 31)*. Supine blood sampling is crucial.

Although seated sampling has been suggested if supine sampling is carried out after positive results *(32)*, this approach entails an overabundance of false-positive results, severely eroding positive predictive value *(33)* and incentive to follow up on positive results *(34, 35)*. As shown here, with supine sampling and appropriately established reference intervals, a positive result for the plasma test can provide probabilities of disease reaching >75% for patients of the low-risk group who show increases of any single plasma metabolite ≥2-fold above UCs, as observed in nearly 99% of patients with disease of that group. When combined with positive results that also include increases in ≥2 metabolites of the panel, as observed in >70% of patients with PPGLs, the probability of disease approaches 100%.

The study has some limitations, including not being population based, a necessity of study design to recruit sufficient numbers of patients with disease (see Discussion section in the online Data Supplement). Other limitations, including that 3% of patients without PPGLs were lost to follow-up and that urinary measurements were not possible in 4% of all patients, are similarly addressed in the Discussion section included in the online Data Supplement.

Despite the above limitations, the study establishes that with appropriate preanalytical precautions, the plasma panel provides superior performance for diagnosis of PPGLs compared with panels of either urinary free or deconjugated metabolites. Among the 2 urinary tests, the advantages of measuring the free over the deconjugated metabolites should fuel efforts to phase out the latter measurements in favor of the former. Although measurements of urinary free metabolites might be advisable when it is not possible to comply with the requirements of the plasma test, it must also be appreciated that urinary methoxytyramine provides negligible diagnostic value compared with plasma measurements, the latter being most important for patients at high risk of PPGLs.

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